

IN THE CLAIMS:

1. (Original): A human feeder cell layer which supports the derivation of ES cells in a substantially undifferentiated state said feeder cell layer comprising cells selected from the group including human adult, fetal or embryonic cells or a combination thereof.
2. (Original): A human feeder cell layer which supports the culture of ES cells in a substantially undifferentiated state said feeder cell layer comprising cells selected from the group including human adult, fetal or embryonic cells or a combination thereof.
3. (Original): A human feeder cell layer according to claim 1 or 2 wherein the human adult cell is selected from the group including human fibroblast cells, human adult skin and human adult muscle fibroblasts and adult epithelial cells or a combination thereof.
4. (Original): A human feeder cell layer according to claim 3 wherein the human adult cell is a human fibroblast cell.

[[4]] 5. (Presently Amended): A human feeder cell layer according to claim 3 or 4 wherein the human fibroblast cell is a human adult fallopian tubal (HAFT) fibroblast cell.

5 6. (Presently Amended): A human feeder cell layer according to claim 3 wherein the human adult cell is a human skin cell.

~~6~~ 7. (Presently Amended): A human feeder cell layer according to claim 3 wherein the human adult cell is a human muscle cell.

~~7~~ 8. (Presently Amended): A human feeder cell layer according to claim 3 wherein the human adult cell is a human adult epithelial cell.

~~8~~ 9. (Presently Amended): A human feeder cell layer according to claim 3 or ~~7~~ 8 wherein the human adult epithelial cell is a human oviductal epithelial cell.

~~9~~ 10. (Presently Amended): A human feeder cell layer according to claim 1 or 2 wherein the human fetal cell is a human fetal muscle (HFM) or human fetal skin (HFS) cell or combination thereof.

~~10~~ 11. (Presently Amended): A human feeder cell layer according to claim ~~9~~ 10 wherein the human fetal cell is a HFM cell.

~~11~~ 12. (Presently Amended): A human feeder cell layer according to claim ~~9~~ 10 wherein the human fetal cell is a HFS cell.

~~12~~ 13. (Presently Amended): A human feeder cell layer according to claim 1 or 2 wherein the human embryonic cell is a human embryonic muscle (HEM) or human embryonic skin cell or combination thereof.

~~13~~ 14. (Presently Amended): A human feeder cell layer according to claim ~~12~~ 13 wherein the human embryonic cell is a HEM cell.

~~14~~ 15. (Presently Amended): A human feeder cell layer according to claim ~~12~~ 13 wherein the human embryonic cell is a human embryonic skin cell.

~~15~~ 16. (Presently Amended): A human feeder cell layer according to ~~any one of claims 1 to 14~~ claim 1 or 2, which is first established in a primary culture in the presence of HFE medium.

~~16~~ 17. (Presently Amended): A human feeder cell layer according to ~~any one of claims 1 to 15~~ claim 16 wherein the feeder layer is propagated in the presence of a HM medium.

~~17~~ 18. (Presently Amended): A human feeder cell layer according to ~~any one of claims 1 to 16~~ claim 1 or 2 comprising fibroblast cell line Detroit 551 (ATCC NO CCL-110).

~~18~~ 19. (Presently Amended): A human feeder cell layer according to ~~any one of claims 1 to 16~~ claim 1 or 2 comprising cell line MRC-5 having accession Number ATCC No. X-55 or ATCC No. CCL-171.

~~19~~ 20. (Presently Amended): A human feeding layer according to ~~any one of claims 1 to 16~~ claim 1 or 2 comprising cell line WI-38 having Accession Number ATCC NO CCL-75 or ATCC NO CCL-75.1.

20 21. (Presently Amended): A method of deriving an embryonic stem (ES) cell line in a substantially undifferentiated state from an ES cell population said method comprising:

obtaining an ES cell population comprising undifferentiated ES cells; and
culturing the undifferentiated ES cells on a cell support matrix in the presence of soluble factors derived from human feeder cells or equivalents thereof.

21 22. (Presently Amended): A method according to claim 20 21 wherein deriving an ES cell line is selected from the group including creating an ES cell line from a source of ES cells and wherein the ES cells are previously uncultured cells; extending propagation or culturing time of an ES cell line wherein the ES cell line is an established cell line; and propagating an established ES cell line.

22 23. (Presently Amended): A method according to claim 20 or 21 22 wherein the deriving of the ES cell line includes propagating an ES cell line.

23 24. (Presently Amended): A method according to ~~any one of claims 20 to 22~~ claim 21 wherein the ES cell population is derived from a source selected from the group including an embryo, blastocyst, inner cell mass (ICM) cells, and a culture of ES cells which have not differentiated.

24 25. (Presently Amended): A method according to claim 23 24 wherein the source is from a blastocyst.

25 26. (Presently Amended): A method according to ~~any one of claims 20 to 24~~ claim 21 wherein the soluble factors are derived from human feeder cells selected from the group including human adult, fetal or embryonic cells or a combination thereof.

26 27. (Presently Amended): A method according to claim 25 26 wherein the human adult cell is selected from the group including human fibroblast cells, human adult skin and human adult muscle fibroblasts and adult epithelial cells or a combination thereof.

27 28. (Presently Amended): A method according to claim 26 27 wherein the human adult cell is a human fibroblast cell.

28 29. (Presently Amended): A method according to claim 26 or 27 wherein the human fibroblast cell is a human adult fallopian tubal (HAFT) fibroblast cell.

29 30. (Presently Amended): A method according to claim 26 27 wherein the human adult cell is a human skin cell.

30 31. (Presently Amended): A method according to claim 26 27 wherein the human adult cell is a human muscle cell.

31 32. (Presently Amended): A method according to claim 26 27 wherein the human adult cell is a human adult epithelial cell.

32 33. (Presently Amended): A method according to claim 26 27 wherein the human adult cell is a human oviductal epithelial fibroblast.

33 34. (Presently Amended): A method according to claim 25 26 wherein the human fetal cell is a human fetal muscle (HFM) or human fetal skin (HFS) cell or combination thereof.

34 35. (Presently Amended): A method according to claim 33 34 wherein the human fetal cell is a HFM cell.

35 36. (Presently Amended): A method according to claim 33 34 wherein the human fetal cell is a HFS cell.

36 37. (Presently Amended): A method according to claim 25 26 wherein the human embryonic cell is a human embryonic muscle (HEM) or human embryonic skin cell or combination thereof.

37 38. (Presently Amended): A method according to claim 36 37 wherein the human embryonic cell is a HEM cell.

38 39. (Presently Amended): A method according to claim 36 37 wherein the human embryonic cell is a human embryonic skin cell.

39 40. (Presently Amended): A method according to ~~any one of claims 20 to 38~~ claim 21 wherein the human feeder cells are cultured in the presence of a medium selected from a group including HES, KO, HF, HES-HS, KO-HS, and HF-HS as hereinbefore described.

40 41. (Presently Amended): A method according to claim 39 40 wherein the medium is HES-HS or KO-HS.

41 42. (Presently Amended): A method according to claim 40 41 wherein the medium is KO-HS.

42 43. (Presently Amended): A method according to ~~any one of claims 20 to 41~~ claim 21 wherein the cell support matrix is a non-cellular cell support matrix selected from the group including Collagen I, Collagen IV, human extracellular matrix or Matrigel or a combination thereof.

43 44. (Presently Amended): A method according to ~~any one of claims 20 to 42~~ claim 43 wherein the cell support matrix comprises Collagen I or Type I Collagen.

[[44]] 45. (Presently Amended): A method of deriving an embryonic stem (ES) cell line in a substantially undifferentiated state from an ES cell population said method comprising:
obtaining an ES cell population comprising undifferentiated ES cells;
and culturing the undifferentiated ES cells on a cell support matrix in the presence of
soluble factors derived from human feeder cells or equivalents thereof, according to any one

~~of claims 20 to 41~~ wherein the cell support matrix comprises a human feeder cell layer according to ~~any one of claims 1 to 19~~ claim 1 or 2.

45 46. (Presently Amended): A method according to ~~any one of claims 20 to 44~~ claim 45 wherein the ES cells are cultured in the presence of a medium selected from the group including HES, KO, HES-HS, KO-HS and HF-HS as hereinbefore described.

46 47. (Presently Amended): A method according to claim 45 46 wherein the medium is KO-HS.

47 48. (Presently Amended): A method according to ~~any one of claims 20 to 46~~ claim 45 wherein the feeder cells are first established in primary cultures in the presence of HFE medium, as hereinbefore described.

48 49. (Presently Amended): A method according to ~~any one of claims 20 to 47~~ claim 45 wherein the feeder cells are propagated in the presence of a HM medium prior to culture with ES cells, as hereinbefore described.

49 50. (Presently Amended): A method according to ~~any one of claims 20 to 48~~ claim 45 wherein the human feeder cell is the fibroblast cell line Detroit 551 (ATCC NO CCL-110).

50 51. (Presently Amended): A method according to ~~any one of claims 20 to 48~~ claim 45 wherein the human feeder cell is the cell line MRC-5 having Accession Number ATCC No. X-55 or ATCC No CCL 171.

51 52. (Presently Amended): A method according to ~~any one of claims 20 to 48~~ claim 45 wherein the human feeder cell is the cell line WI-38 having Accession Number ATCC-CCL-75 or ATCC-CCL-75.1.

52 53. (Presently Amended): A method according to ~~any one of claims 20 to 51~~ claim 45 wherein the ES cell line is cultured in the absence of LIF.

53 54. (Presently Amended): A cellular composition comprising proliferating undifferentiated ES cells and wherein the cell composition comprises the propagated or derived ES cells prepared by the methods according to ~~any one of claims 20 to 52~~ claim 21.

54 55. (Presently Amended): An undifferentiated ES cell line prepared by a method according to ~~any one of claims 20 to 52~~ claim 21.

55 56. (Presently Amended): A cell culture system for deriving and culturing ES cells in a substantially undifferentiated state, said culture system including:

a cell support matrix; and
a cell culture medium for providing soluble factors derived from a human feeder cell selected from the group including a human adult, fetal or embryonic cell.

~~56~~ 57. (Presently Amended): A cell culture system according to claim ~~55~~ 56 wherein the human adult cell is selected from the group including human adult fallopian tubal (HAFT) fibroblast cells, human adult skin and human adult muscle fibroblasts and adult epithelial cells or a combination thereof.

~~57~~ 58. (Presently Amended): A cell culture system according to claim ~~56~~ 57 wherein the human adult cell is a human fibroblast cell.

~~58~~ 59. (Presently Amended): A cell culture system according to claim ~~56~~ or 57 wherein the human adult cell is a human adult fallopian tubal (HAFT) fibroblast cell.

~~59~~ 60. (Presently Amended): A cell culture system according to claim ~~56~~ 57 wherein the human adult cell is a human skin cell.

~~60~~ 61. (Presently Amended): A cell culture system according to claim ~~56~~ 57 wherein the human adult cell is a human muscle cell.

~~61~~ 62. (Presently Amended): A cell culture system according to claim ~~56~~ 57 wherein the human adult cell is a human adult epithelial cell.

~~62~~ 63. (Presently Amended): A cell culture system according to claim ~~56~~ 57 wherein the human epithelial adult cell is a human oviductal epithelial cell.

~~63~~ 64. (Presently Amended): A cell culture system according to claim ~~55~~ 56 wherein the human fetal cell is a human fetal muscle (HFM) or human fetal skin (HFS) cell or combination thereof.

~~64~~ 65. (Presently Amended): A cell culture system according to claim ~~63~~ 64 wherein the human fetal cell is a HFM cell.

~~65~~ 66. (Presently Amended): A cell structure system according to claim ~~63~~ 64 wherein the human fetal cell is a HFS cell.

~~66~~ 67. (Presently Amended): A cell culture system according to ~~55~~ 56 wherein the human embryonic cell is a human embryonic muscle (HEM) or human embryonic skin cell or combination thereof.

~~67~~ 68. (Presently Amended): A cell culture system according to claim ~~66~~ 67 wherein the human embryonic cell is a HEM cell.

~~68~~ 69. (Presently Amended): A cell culture system according to claim ~~66~~ 67 wherein the human embryonic cell is a human embryonic skin cell.

~~69~~ 70. (Presently Amended): A cell culture system according to claim ~~55~~ 56 wherein the cell support matrix comprises Collagen I or matrigel or a combination thereof.

~~70~~ 71. (Presently Amended): A cell culture system according to claim ~~69~~ 70 wherein the cell support matrix comprises Collagen I.

~~71~~ 72. (Presently Amended): A cell culture system according to ~~any one of claims 55 to 70~~ claim 56 wherein the cell culture medium is a conditioned medium including soluble factors derived from a human feeder cell layer.

~~72~~ 73. (Presently Amended): A cell culture system ~~according to claim 55 to 68 for deriving and culturing ES cells in a substantially undifferentiated state, said culture system including:~~
a cell matrix; and

a cell culture medium for providing soluble factors derived from a human feeder cell selected from the group including a human adult, fetal or embryonic cell, wherein the cell support matrix comprises a human feeder cell layer according to any one of claims 1 to 14 claim 1 or 2.

~~73~~ 74. (Presently Amended): A cell culture system according to ~~any one of claims 55 to 72~~ claim 56 wherein the culture medium is selected from the group including HES, KO, HES-HS, and KO-HS.

~~74~~ 75. (Presently Amended): A cell culture system according to claim ~~73~~ 74 wherein the medium is KO-HS.

75 76. (Presently Amended): A conditioned medium for deriving and culturing an ES cell line in a substantially undifferentiated state said medium prepared by a method including:

obtaining a feeder cell layer according to ~~any one of claims 1 to 19~~ claim 1 or 2;

culturing the feeder cells in the presence of a medium selected from the group including HES, KO, HES-HS, KO-HS, HFE, HM, HF or HF-HS; and

separating the medium from the cells to obtain conditioned medium.

76 77. (Presently Amended): A conditioned medium according to claim 75 76 wherein the human feeder cell layer comprises adult skin cells.

77 78. (Presently Amended): A conditioned medium according to claim 76 77 wherein the human feeder cell layer comprises HFM cells.

78 79. (Presently Amended): A conditioned medium according to ~~any one of claims 75 to 77~~ claim 76 wherein the medium comprises KO-HS.